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REVIEW

Interdependent roles of BRCA1, CtBP, estrogen, and NADH/NAD+ ratio in error-prone repair, breast cancer initiation, and metastasis

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Abstract

Genomic instability may result from a shift in the double-strand break (DSB) repair pathway from homologous recombination (HR) to error-prone non-homologous end joining (NHEJ). Normal BRCA1 expression is essential for high-fidelity HR, and its deficiency may promote error-prone NHEJ. Similarly, a low NADH/NAD+ ratio promotes low-fidelity HR, whereas a high NADH/NAD+ ratio promotes NHEJ. Further, although p53 inhibits HR, it is required for the high fidelity of this process. Furthermore, estrogen promotes NHEJ and nuclear export of p53, leading to low-fidelity HR or error-prone NHEJ. Thus, a shift in BRCA1 expression, NADH/NAD+ ratio, or a higher level of estrogen may cause genomic instability, which may initiate breast cancer. Furthermore, hypoxia may shift DSB repair from HR to NHEJ by repressing BRCA1 through dimeric CtBP, which forms under an elevated NADH/NAD+ ratio caused by hypoxia. Genomic instability caused by this shift in the DSB repair mechanism under hypoxia promotes EMT-induced breast cancer metastasis. This review discusses the roles of CtBP, BRCA1, estrogen, and metabolic shift linked to an altered NADH/NAD+ ratio in the initiation of breast cancer and EMT-mediated metastasis.

Keywords Breast cancer initiation · Genomic instability · Homologous recombination · Nonhomologous end joining · CtBP · BRCA1 · Estrogen · EMT · Metastasis

Introduction

Dimeric CtBP is an NADH-dependent corepressor that requires NADH for dimerization [1–5]. Immunohistochemical analysis of human breast cancer tissue showed that nearly 92% of invasive ductal breast cancer cases display CtBP1-positive staining, whereas only about 4% of normal breast tissue samples do so [6]. CtBP1 causes breast cancer progression by regulating the NF-κB pathway [7]. Furthermore, CtBP2 is overexpressed in ovarian carcinoma and represses BRCA1, a core protein involved in DNA damage repair [8, 9]. Similar to CtBP2, CtBP1 represses BRCA1 expression [6]. In aggressive breast cancer subtypes, CtBP-repressed genes are specifically downregulated, and higher CtBP levels are associated with worse clinical outcomes and lower median survival [10]. Consistently, 57% of human invasive ductal breast cancers have a loss of BRCA1 [6], which is repressed by dimeric CtBP overexpression in breast cancer. Comprehensive analyses of the role of CtBP in breast cancer development indicate that it plays a key role in regulating stem cell pathways, EMT, and genomic instability [10]. Similarly, BRCA1 loss promotes EMT in breast cancer through TGFβR2 pathway activation [11].

In eukaryotes, DNA double-strand breaks (DSBs) are primarily repaired through two pathways: NHEJ and HR [12, 13]. NHEJ requires the Ku70-Ku80 DNA end-binding complex, whereas HR requires the Mre11-Rad50-Nbs1 (MRN) complex [12]. NHEJ is preferred during the pre-replicative phase of the cell cycle, whereas HR is preferred during the post-replicative phase [12]. BRCA1 is crucial for HR. It colocalizes with the MRN complex and Rad50, both of which are necessary for HR [14, 15]. Furthermore, BRCA1 promotes CtIP-mediated DNA end resection during DSB repair [16, 17]. DNA end resection inhibits NHEJ and commits cells to HR. Furthermore, BRCA1 transactivates p21 upon DNA damage [18, 19], causing cell cycle arrest and allowing the HR-mediated repair process to be completed.

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There is growing evidence that error-prone NHEJ may play a role in carcinogenesis and genomic instability [20, 21]. NHEJ is considered the dominant mechanism for the repair of DSBs in the G0 and G1 phases of the cell cycle [20, 22]. Thus, quiescent stem cells may be prone to genomic instability because they primarily rely on NHEJ, an inherently less accurate DSB repair pathway. Consistently, NHEJ promotes the acquisition of genomic rearrangements and mutagenesis in quiescent HSCs [21]. In this context, the accuracy of canonical NHEJ, which is only partially error-prone, is controlled by Ku80, and in its absence, mutagenic microhomology-mediated repair occurs efficiently [23].

Furthermore, altered metabolism is involved in breast cancer development [24]. In this review, we implicate the altered expression of BRCA1 and CtBP, a shift in the NADH/NAD+ ratio, and estrogen levels in breast cancer initiation and metastasis through error-prone NHEJ or low-fidelity HR.

BRCA1 deficiency may activate the error-prone NHEJ pathway

The role of BRCA1 in determining the DSB repair pathway is complex. Instead of acting as a single unit, BRCA1 functions within at least three different multiprotein complexes, namely BRCA1-A, BRCA1-B, and BRCA1-C. Each of these three performs a specific role in the DNA damage response network [25]. Notably, when the cell cycle is in the S and G2 phases [26], BRCA1 mainly promotes high-fidelity HR by promoting DNA end resection dependent on CtIP, following phosphorylation of CtIP on Ser327 [27].

BRCA1 deficiency decreases the frequency of HR and increases the frequency of NHEJ [28, 29]. HR is Rad51/RPA dependent [30], whereas NHEJ is DNA-PK-dependent [26, 31, 32]. Furthermore, RIF1 is required for 53BP1-dependent NHEJ and inhibits HR [33]. On the other hand, RIF1 is antagonized by BRCA1 in S-phase to switch the DSB repair to HR [33]. In this context, BRCA1 recruits UHRF1, an E3 ubiquitin ligase, which is phosphorylated by BRCA1 at Ser674 in S-phase. Subsequently, UHRF1 causes K63-linked polyubiquitination of RIF1, dissociating it from 53BP1 and DSB, initiating HR during S and G2 phases [34]. Further, ATM-BRCA1 inhibits error-prone NHEJ [35], thereby promoting the fidelity of DSB repair. Thus, BRCA1 promotes HR and inhibits NHEJ, and its normal expression may affect the relative frequency of HR and NHEJ. In contrast, during the replicative stages of the cell cycle, hypoxia suppresses BRCA1 expression by dynamically redistributing E2F-promoter occupancy and dimerizing CtBP, which represses BRCA1, impairs HR, and shifts the DSB repair process to NHEJ [36]. Thus, BRCA1 is essential for high-fidelity HR, and its repression may increase the frequency of error-prone NHEJ (Fig. 1).

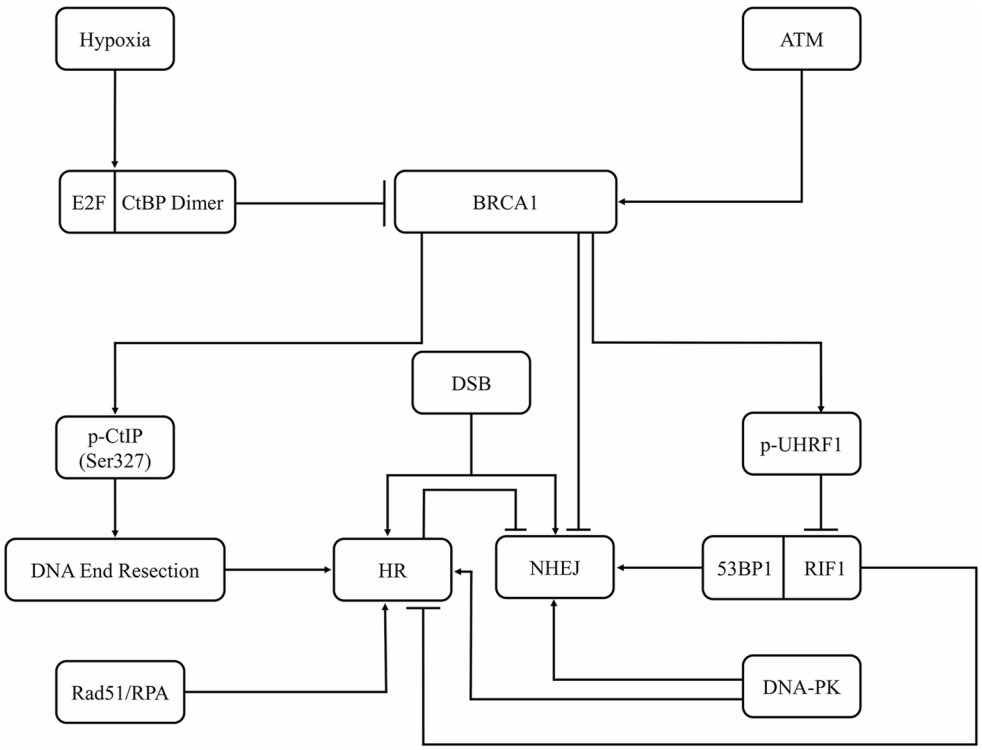

Fig. 1 BRCA1 controls the switch in the DNA repair pathway. BRCA1 is activated by ATM during S/G2 phases of the cell cycle, promoting HR and inhibiting the error-prone NHEJ pathway. In contrast, when hypoxia inhibits BRCA1 via the E2F or CtBP dimer, it promotes error-prone NHEJ, driven by 53BP1, RIF1, and DNA-PK. The figure was created in Microsoft PowerPoint

A higher NADH/NAD+ ratio promotes error-prone NHEJ, and a lower NADH/NAD+ ratio promotes low-fidelity HR

End resection and HR are impaired in CtIP (CtBP-interacting protein) mutants that are unable to form dimers, indicating that CtIP dimerization is crucial for its role in DSB repair following DNA damage [37, 38]. CtBP monomers are necessary for the formation of CtIP dimers that participate in the HR process because CtIP binds to CtBP monomers [37]. Consistently, depletion of CtBP1/2 monomers shifted the DSB repair pathway from HR to NHEJ and caused apoptosis in high-grade serous ovarian carcinoma cells, suggesting that CtBP monomers promote HR [39]. In contrast, CtBP dimers inhibit HR by repressing BRCA1 expression. BRCA1 assists CtIP in HR, and CtBP dimers repress BRCA1 in an NADH-dependent manner [40]. Thus, CtBP dimers, which form when the NADH/NAD+ ratio is high, shift the DSB repair pathway from HR to NHEJ by repressing BRCA1 when the NADH/NAD+ ratio is high (Fig. 2A). During the repair process, PARP1 induces NAD+ depletion, increasing the NADH/NAD+ ratio, decreasing glycolysis, and increasing oxidative phosphorylation, thereby maintaining cell survival [41]. Furthermore, PARP1 regulates the balance between HR and NHEJ by preventing excessive NHEJ [42]. In this context, PARP1 competes with Ku and inhibits excessive NHEJ [43]. The binding of NAD + to DBC1 prevents it from inhibiting PARP1 [44]. Thus, under higher NADH/NAD+, PARP1 is inhibited by DBC1, and DSBs are repaired by the hyperactive NHEJ pathway. Furthermore, a higher NADH/NAD+ ratio inactivates SIRT1, another NAD+-dependent protein. Since SIRT1 promotes HR [45], the increase in the NADH/NAD+ ratio further promotes hyperactive NHEJ by deactivating SIRT1.

Conversely, when the NADH/NAD+ ratio decreases, CtBP shifts to its monomeric form. This leads to CtIP dimerization. CtIP dimers, together with BRCA1, promote the HR pathway [16]. Additionally, at a lower redox ratio (i.e., NADH/NAD+ ratio), CtBP monomers inhibit p53 [46], which in turn inhibits HR through its interaction with RPA [47]. Both wild-type and transactivation-deficient p53 mutants inhibited HR by sequestering RPA and preventing its binding to ssDNA [48]. Furthermore, p53 interacts with RAD51 and RAD54, and HR inhibition by p53 depends on

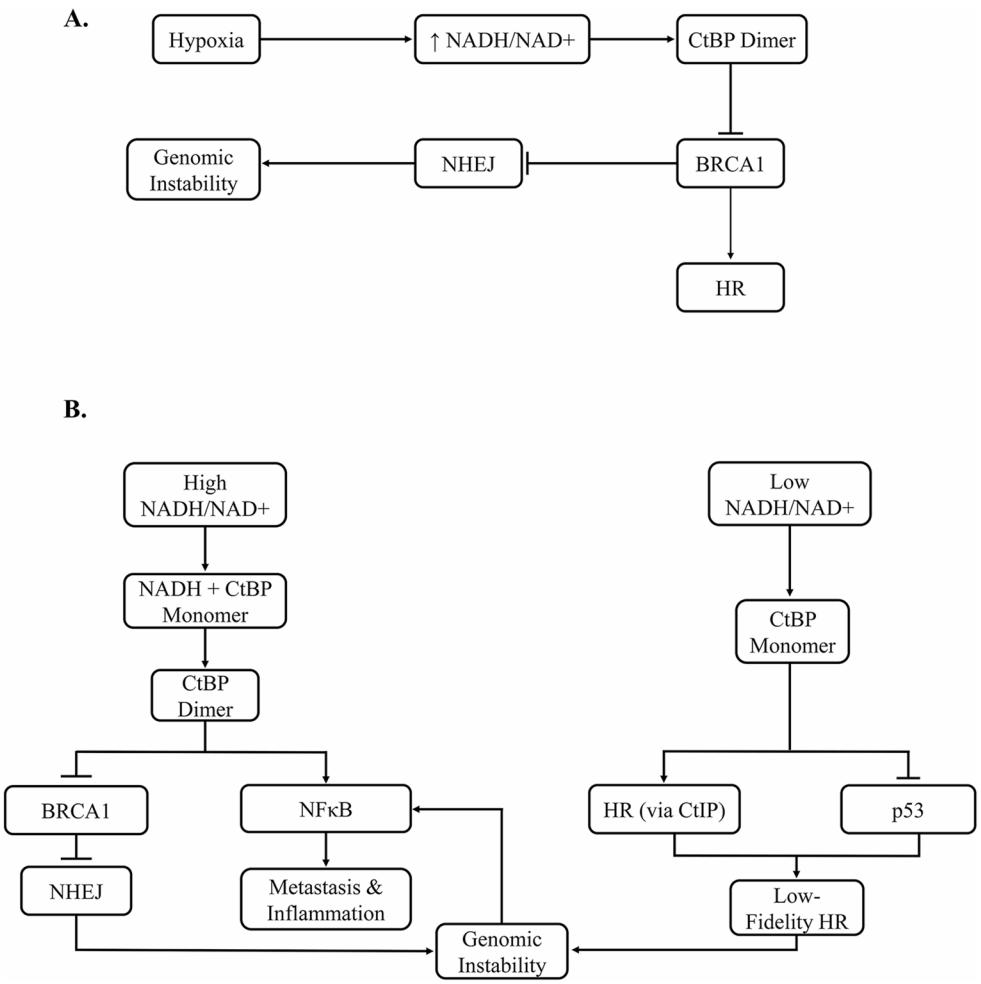

Fig. 2 Role of CtBP – BRCA1–metabolism feedback loop in the DNA repair process. (A) CtBP links metabolism to DNA repair via the NADH/NAD+ ratio. Dimeric CtBP, which forms under a higher NADH/NAD+ ratio, represses BRCA1 and HR, whereas monomeric CtBP, which forms under a lower NADH/NAD+ ratio, promotes CtIP-dependent HR. (B) Effect of altered NADH/NAD+ ratio on DSB repair. The figure was created in Microsoft PowerPoint

its direct binding to RAD51 [49]. Similarly, BLM helicase prevents RAD51 loading at DSB sites and prevents excessive HR [50]. In contrast, BLM also promotes HR by processing Holliday junctions [51, 52]. In this context, p53 maintains a balance between BLM- and RAD51-mediated pathways by interacting with them, preventing excessive HR and maintaining the high fidelity of the process [53]. Thus, p53 can inhibit HR and increase its fidelity in multiple ways. In contrast, a lower redox ratio promotes HR by inhibiting p53 via CtBP monomer activity. However, HR at lower redox ratios is less accurate because high-fidelity HR requires p53, which is impaired at lower redox ratios [46]. Therefore, both an increase and a decrease in the redox ratio can cause breast cancer initiation (Fig. 2B). Consistently, lower activity of mitochondrial complex I increases the NADH/NAD+ ratio and aggressiveness of breast cancer, while restoring the tumor cell NADH/NAD+ balance reduces tumor cell growth and metastasis [54]. Similarly, a lower-than-normal NADH/NAD+ ratio has been linked to more invasive breast cancer cells [55]. Thus, a higher NADH/NAD+ ratio promotes error-prone NHEJ, whereas a lower NADH/NAD+ ratio promotes low-fidelity HR, leading to genomic instability and cancer.

Furthermore, BRCA1 makes a complex with PALB2-BRCA2 and regulates the loading of RAD51 at the DNA break in the HR repair process [56], while CtBP helps make CtIP dimers, which, with the help of BRCA1, participate in the HR repair process. Thus, both PALB2 and CtBP are involved in BRCA1 dependent HR pathway. In contrast, BRCA2-PALB2 may also load RAD51 at DNA breaks in a BRCA1-independent manner [57]. Thus, under a higher NADH/NAD+ ratio, when CtBP represses BRCA1, BRCA1-independent RAD51 loading via PALB2-BRCA2 may promote HR; however, this HR may be error-prone in the absence of BRCA1 [58].

Estrogen may switch the DSB repair pathway from HR to NHEJ

Estrogens also generate reactive oxygen species that can damage DNA [59]. In mammary cells, the loss of CtBP from the BRCA1 promoter via estrogen induction raises BRCA1 expression [40], which may shift the DSB repair pathway from HR to NHEJ. In this context, while normal levels of BRCA1 promote HR through CtIP, BRCA1 overexpression inhibits the MRN complex's exonuclease activity [15] and facilitates RPA removal in the S and G2 phases [26], thereby inhibiting HR. In agreement, breast cancer cells treated with 17β-estradiol (E2) showed a significant reduction in their capacity to metabolize peroxides and an increase in their sensitivity to DNA damage [60], suggesting a role for the error-prone NHEJ pathway in DSB repair under estrogen treatment. These effects were not observed in estrogen receptor alpha (ERα)-negative cells [60]. Thus, in breast cancer cells, antioxidant status is modulated by ER [60], which relieves CtBP-mediated repression of BRCA1, leading to BRCA1 overexpression and a shift in the DSB repair pathway from HR to error-prone NHEJ (Fig. 3). Furthermore, ERα modulates DNA-PK expression, a key protein involved in NHEJ [61]. On the other hand, estrogen inhibits ATM expression [62], a key protein involved in HR [63]. Furthermore, estrogen positively regulates several key components of the NHEJ pathway [64]. Since estrogen increases DNA-PK expression, and DNA-PK phosphorylates ERα at Ser-118 [65], thereby increasing ERα stability, estrogen and DNA-PK form a positive feedback loop that reinforces the NHEJ pathway. Thus, there are multiple ways estrogen increases the frequency of the NHEJ pathway.

Breast cancer that expresses estrogen receptor has wild-type p53 and shows a good prognosis in the absence of estrogen

In response to DNA damage, p53 is activated, causing cell cycle arrest and DNA repair [66]. p53 has been found to be mutated in only 20% of breast cancers, whereas ERα is expressed in 70% of all breast cancer cases [66]. ERα

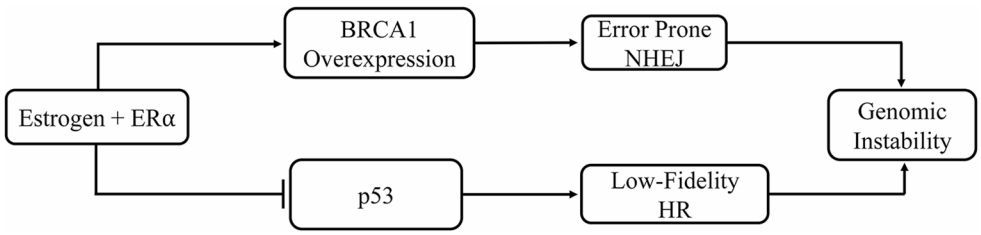

Fig. 3 Estrogen's dual action. By relieving CtBP-mediated repression of BRCA1, the ER signaling pathway upregulates BRCA1, biasing DSB repair toward NHEJ. Additionally, ER induces p53 nuclear exclusion and oxidative stress, thereby decreasing HR fidelity. The figure was created in Microsoft PowerPoint

activates p53 transcription [66]. Since breast cancers positive for ER have wild-type p53, they respond to antiestrogen therapy [66]. In contrast, p53 is frequently mutated in ER-negative breast cancer cells [67]. Furthermore, ERα depletion in ER-positive breast cancer patients causes aggressive tumor growth, tumor invasion, and poor outcomes [66] since in these cells, wild-type p53 function is reduced. Furthermore, ERα loss desensitizes breast cancer cells to growth suppression induced by DNA damage because wild-type p53 function is lost in these cells [66]. Thus, wild-type p53 expression in ER+ breast cancer cells reduces tumor aggressiveness. However, the effects of ER/p53 are only observed in the absence of estrogen. In the presence of estrogen, ER+ breast cancers are resistant to chemotherapy-induced apoptosis [67]. Furthermore, positive modulators of ER activity, such as estradiol and tamoxifen, antagonize p53 [67]. In contrast, ER antagonism by fulvestrant induces p53-mediated apoptosis [67]. Thus, treatment of ER+ breast cancer with ER antagonists may be a potent anti-cancer strategy. Further, similar to the transcriptional regulation of p53 by ERα, p53 binds to the ER promoter and regulates its transcription, accounting for the concurrent expression of p53 and ERα [68]. Besides regulating p53 transcription, ERα directly binds p53 and recruits corepressors, NCoR and SMRT, as well as histone deacetylase 1 (HDAC1), thereby suppressing p53's transactivation activity [69]. In this context, 17β-estradiol (E2) increases the binding between p53 and ERα, repressing p21, a target gene of p53, whereas antiestrogens decrease the binding between p53 and ERα, inducing p21 transcription [69]. The effect of estrogen and antiestrogens on p53 target genes was opposite to their effect on pro-proliferation ERE-containing target genes of ERα [69]. Thus, ERα uses a dual strategy in cancer: transcriptionally activating ERE-containing proliferative genes and repressing p53-responsive anti-proliferative genes [69].

Although ER-positive breast cancer cells express wild-type p53, p53 is translocated to the cytoplasm in the presence of estrogen in these cells

In MCF-7 cells, p53 is predominantly localized to the nucleus, whereas treatment with estrogen causes the localization of p53 to the cytoplasm [70]. Thus, estradiol can inactivate wild-type p53 in ER+ breast cancer cells by nuclear exclusion, enabling cyclin-dependent phosphorylation events that drive cell cycle progression [70]. Moreover, activation of ER signaling enhances the suppression of p53 activity, whereas blocking ER prevents interference with p53-driven cell death [67]. Consequently, although estrogen receptor-positive breast cancer cells retain wild-type p53, estrogen-induced cytoplasmic translocation of p53 renders these cells less responsive to chemotherapy-induced apoptosis.

p53 interacts with RPA and inhibits HR [71]. Although p53 inhibits HR, it is required for the fidelity of this process [72]. Thus, in normal cells, estradiol-induced p53 inactivation may contribute to tumorigenesis in an estrogen-dependent manner [70], since estrogen induces the error-prone NHEJ pathway and decreases the fidelity of HR by the nuclear expulsion of p53 (Fig. 3).

DNA-PK activity is required for efficient HR during the replicative phases of the cell cycle

p53 interacts with RPA and suppresses HR under normal conditions [71]. RPA is phosphorylated by DNA-PK, whereas ATM or ATR phosphorylates p53. This dual modification disrupts the p53-RPA interaction, freeing RPA to participate in HR [71]. Thus, DNA-PK activity promotes HR. In contrast, in NHEJ, DNA-PK protects DNA ends from unwarranted DNA processing [73, 74]. However, for the completion of the DNA repair process, the ends need to become accessible, which occurs when DNA-PK is inactivated by autophosphorylation at Thr2609 [73–76]. Thus, phosphorylation of DNA-PK at the Thr2609 position promotes the NHEJ pathway [77]. In HR, DNA-PK activity is important for the progression of mitosis and chromosomal stability through Chk2-BRCA1 [78], while the MRN complex and CtIP depend on DNA-PK activity for efficient processing and resection of DNA ends [79]. Thus, DNA-PK activity is important for HR progression and chromosomal stability during the replicative phases of the cell cycle, whereas DNA-PK inhibition via autophosphorylation promotes NHEJ activity.

CtBP inhibits cholesterol synthesis and promotes epithelial-to-mesenchymal transition (EMT) of breast cancer cells

EMT is required for metastasis to occur [80, 81]. The TGF-β pathway triggers this transition in breast cancer cells [80, 82]. In this context, cholesterol acts as a negative regulator by reducing TGF-β receptor stability [80]. Sterol regulatory element binding protein-2 (SREBP2), a transcription factor that controls cholesterol biosynthesis, is encoded by the SREBF2 gene [83]. In breast cancer cells, CtBP partners with ZEB1 to form a repressive complex that downregulates SREBP2 expression [80]. Thus, CtBP negatively regulates cholesterol synthesis. Since cholesterol negatively regulates TGF-β receptors, which are involved in the EMT of breast

cancer cells, CtBP, which reduces cholesterol, induces EMT by increasing the stability of TGF-β receptors. Conversely, TGF-β increases SREBF2 repression via CtBP-ZEB1, thereby lowering cholesterol synthesis and promoting breast cancer metastasis [80]. Thus, a positive feedback loop involving SREBP2 is formed between TGF-β signaling and cholesterol synthesis (Fig. 4A). Consistently, analysis of publicly available breast cancer datasets revealed a negative correlation between CtBP and SREBP2 expression [80]. Furthermore, high CtBP levels and low SREBP2 levels were significantly correlated with tumor EMT [80]. Thus, CtBP represses cholesterol synthesis, stabilizes TGF-β receptors, and promotes EMT in primary tumors (Fig. 4A).

SIRT6 and CtBP-ZEB1 decrease cholesterol, cause EMT, induce autophagy, and promote cancer-stem-cell (CSC) characteristics

Autophagy is necessary for the survival of EMT cells during migration and dissemination [84]. Indeed, TGF-β-induced EMT depends on autophagy-dependent energy metabolism [85]. Disseminated dormant breast cancer cells depend on autophagy for survival [86]. Cancer cells use autophagy to survive various stresses during dissemination, including hypoxia, endoplasmic reticulum (ER) stress, and EMT [87]. Moreover, autophagy in cancer cells may contribute to tumor dormancy and drug resistance [87]. Furthermore,

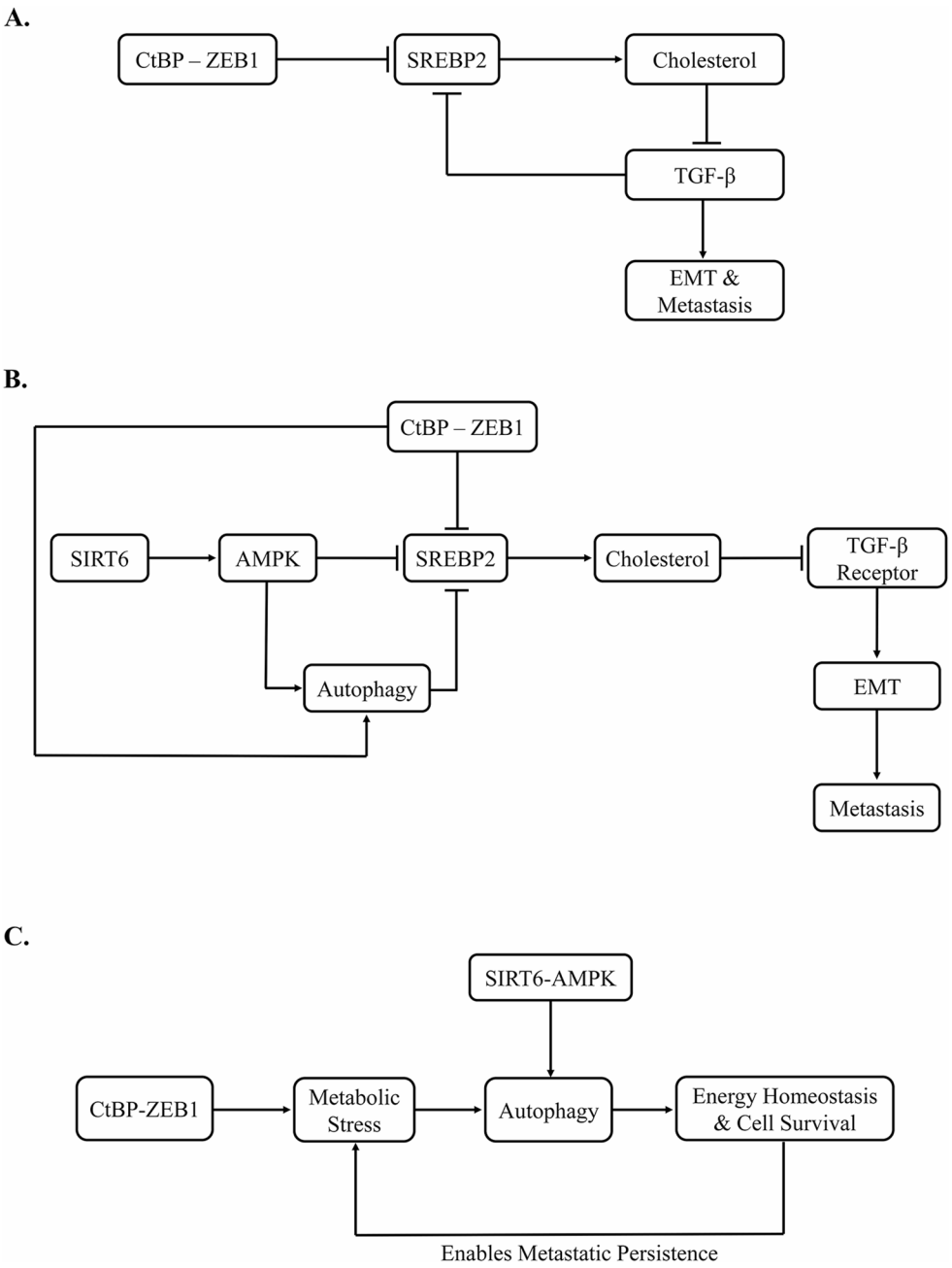

Fig. 4 Feedback Loop controlling EMT and metastasis. (A) CtBP–ZEB1 suppresses SREBP2, thereby reducing cellular cholesterol levels. This, in turn, stabilizes TGF-β receptors, establishing a positive feedback loop between cholesterol and TGF-β that facilitates EMT and metastasis in breast cancer cells. (B) SIRT6 activates AMPK to enhance autophagy and suppress SREBP2, whereas CtBP–ZEB1 and autophagy also repress SREBP2, leading to reduced cholesterol and stabilized TGF-β receptors, which drive EMT and metastasis. (C) EMT-Autophagy Metastatic Survival Circuit-Metastatic success requires a synergistic interplay between EMT and autophagy. Complexes such as CtBP-ZEB1 drive EMT, generating metabolic stress. This triggers the pro-survival autophagy program, which is stimulated by the SIRT6-AMPK pathway. Autophagy enables cancer cells to overcome the metastasis hurdle and survive the metastatic cascade by maintaining energy homeostasis and supporting survival. The figure was created in Microsoft PowerPoint

autophagy plays a crucial role in cell survival during antiestrogen challenge and in the development of antiestrogen resistance [88].

SIRT6 overexpression reduces cholesterol levels and inhibits SREBP2 expression and processing [89]. Thus, SIRT6 promotes EMT by reducing cholesterol levels (Fig. 4B). Furthermore, SIRT6 activates AMPK [90]. AMPK promotes cellular energy reserves by stimulating ATP-producing catabolic processes and suppressing ATP-consuming anabolic activities. By increasing autophagic flux, AMPK activation is crucial for maintaining cellular homeostasis and preventing oxidative stress-induced senescence [91]. Furthermore, cellular activation of AMPK directly inhibits SREBP2 expression [92]. Thus, EMT induces autophagy via the SIRT6-AMPK pathway, and autophagy, in turn, promotes EMT by inhibiting SREBP2 expression. Therefore, EMT and autophagy form an axis that regulates cancer metastasis. Consistently, ZEB1-CtBP, which inhibits cholesterol synthesis and thus promotes EMT, also promotes autophagy [93] (Fig. 4B, C). Furthermore, EMT-autophagy-cancer stem cell characteristics form an axis in the metastasis process [94]. Consistently, the overexpression of SIRT6, which activates AMPK to promote autophagy, has been linked to cancer stem cell (CSC) characteristics, such as tumor dormancy and low senescence [95]. In HER2-positive breast cancer, elevated SIRT6 levels result in a poor prognosis and a high risk of metastasis [95] (Fig. 4B).

Interestingly, SIRT6 is optimized to facilitate more efficient DSB repair in long-lived organisms [96]. SIRT6 stimulates DSB repair via both NHEJ and HR by activating PARP1 under oxidative stress [97]. Similarly, JNK phosphorylates SIRT6 and promotes DSB repair by recruiting PARP1 in response to oxidative stress [98]. Furthermore, SIRT1 deacetylates SIRT6, which is required for SIRT6 polymerization and recruitment to DSBs [99]. Thus, synergy between SIRT1 and SIRT6 is required for DSB repair [99]. However, under hypoxic conditions of a solid tumor, BRCA1 is repressed by CtBP, and SIRT1 is deactivated due to the higher NADH/NAD+ ratio (Reductive stress). Since BRCA1 and SIRT1 are required for HR, under the higher NADH/NAD+, HR is inhibited. Furthermore, PARP1 prevents excessive NHEJ [42]. The higher NADH/NAD+ ratio inhibits PARP1, potentially leading to hyperactive NHEJ. Thus, in the hypoxic environment of solid tumors, HR may be inhibited, and hyperactive NHEJ may occur, leading to genomic instability. Furthermore, genomic instability and EMT are linked. In normal cells, E2F1 promotes HR and genomic stability [100]. In cancer cells, E2F1 transactivates NHEJ factors, including Artemis, DNA-PK, Ku70/80, and NHEJ1, and inhibits the chromatin modifier APLF, which is required for proper NHEJ-mediated DSB repair, leading to errors in DSB repair and increased cancer invasiveness [100].

On the other hand, EMT transcription factors bind to RAD51 promoter regions and promote HR [101]. Furthermore, DNA damage induces EMT in a PARP1-dependent manner [101]. Thus, in cancer, both HR and NHEJ may occur depending on the availability of oxygen and NAD+. While error-prone NHEJ may lead to genomic instability and EMT under a higher NADH/NAD+ ratio in the hypoxic environment of solid tumors, EMT may facilitate successful metastasis by enabling HR-mediated DNA repair in blood vessels and at secondary sites under higher oxygen and NAD+ availability. When oxygen and NAD + are available, SIRT1 is active, deacetylating and inactivating p53 [102], thereby decreasing HR fidelity and preventing apoptosis during DNA repair. Thus, cancer cells may continue to evolve due to defective NHEJ and HR.

Further, p53 suppresses SIRT1 expression by blocking c-Myc binding to the SIRT1 promoter [103]. Thus, p53 and SIRT1 form a double-negative feedback loop: when one is high, the other is low. This may regulate the balance between their activities, establishing a balance between fidelity and HR progression. In cancer cells, this balance may be tilted toward lower fidelity and greater HR progression during EMT and metastasis.

Genomic instability, a key to breast cancer metastasis, is mediated by dimeric CtBP under a high redox ratio

In breast cancer, continuous proliferation of cells undergoing EMT leads to genomic instability [104]. Chromosome mis-segregation during anaphase is evident in chromosomally unstable cells, which is an important factor in tumor development and metastasis [105]. Chromosome segregation errors result in the formation of micronuclei, which can burst and release genomic DNA into the cytoplasm. This activates the cGAS–STING system, a cytosolic DNA sensor, and the downstream non-canonical NF-κB signaling pathway [105], which triggers the innate immune response. Thus, genomic instability drives cancer cell metastasis by co-opting the long-term activation of innate immune pathways [105] (Fig. 5A).

In this context, a higher NADH/NAD+ ratio or CtBP dimerization activates NF-κB signaling, the innate immune response, and the expression of pro-inflammatory genes [106], which are important for EMT and metastasis (Fig. 5A). NADH, owing to a higher redox ratio in the hypoxic environment, promotes CtBP dimerization and corepression activities [4], including the repression of BRCA1. Furthermore, hypoxic conditions, which increase the redox ratio,

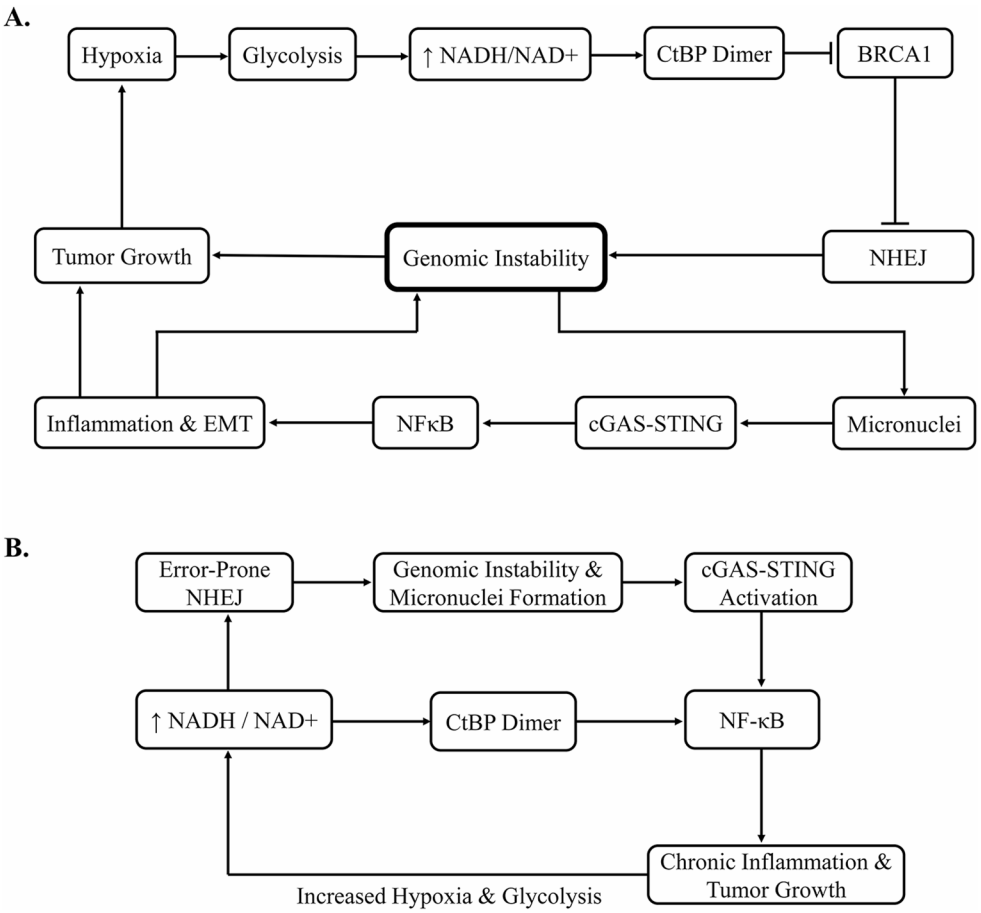

Fig. 5 Metabolic Reprogramming – DNA Repair – Inflammation – EMT and Hypoxia Loop. (A) Hypoxia and metabolic reprogramming increase the NADH/NAD+ ratio, promoting CtBP dimerization and BRCA1 repression. This results in repair by error-prone NHEJ, causing genomic instability and activating innate immunity via NF-κB, which promotes tumor growth by increasing hypoxia and glycolysis, further increasing the NADH/NAD+ ratio in a feedback loop. (B) Instability-Inflammation Feedback Loop: A vicious cycle between EMT-metastasis and genomic instability, inflammation, and NFκB-mediated innate immunity exists, driving cancer evolution and progression. The figure was created in Microsoft PowerPoint

downregulate BRCA1 via E2F and redirect DSB repair from HR to NHEJ [36], thereby causing genomic instability. Furthermore, the dimeric form of CtBP activates innate immunity by activating NF-κB signaling [106]. NF-κB interacts with CtIP-BRCA1 complexes and promotes HR [107]. However, in the hypoxic environment of solid tumors, the higher redox ratio and CtBP dimerization repress BRCA1. Similarly, non-canonical NF-κB proteins p100/52 regulate RAD51 expression and promote HR without significantly affecting NHEJ [108]. However, RAD51 is repressed by E2F4/p130 complexes, decreasing HR and causing genomic instability under hypoxic conditions [109, 110]. Thus, under a higher NADH/NAD+ ratio and hypoxic conditions, NF-κB's ability to promote HR may be limited. Furthermore, NF-κB activity may promote NHEJ by activating Ku70 and Ku80 expression in the presence of COX-2 [111]. Interestingly, COX-2 expression is an important factor in EMT and breast cancer invasiveness [112]. Furthermore, HER2 and COX-2 form a positive feedback loop that amplifies their expression [113]. Moreover, HER2 activates NF-κB [114], thereby promoting the NHEJ pathway and invasiveness. Thus, a higher frequency of NHEJ in the hypoxic environment of solid tumors can be linked to invasiveness. In summary, dimeric CtBP, formed under a higher redox ratio, promotes EMT and causes genomic instability by shifting the DSB repair process to the error-prone NHEJ and co-opts innate immunity by activating the NFκB pathway. Furthermore, error-prone NHEJ is the primary DSB repair mechanism in the hypoxic environment of solid tumors, driving genomic instability that propels tumor evolution and metastasis by triggering EMT and NF-κB-mediated innate immunity via dimeric CtBP (Fig. 5A and B). Consistent with these findings, lowering the redox ratio when elevated suppresses breast cancer metastasis [54] by decreasing dimeric CtBP concentration.

Conclusion

Although BRCA1 is classically known as a tumor suppressor, its severe dysregulation can disrupt repair fidelity, leading to genomic instability and carcinogenesis. Similarly, alterations in the redox ratio contribute to genomic instability. For example, when this ratio increases due to decreased mitochondrial complex I activity, double-strand break repair tends to favor the more error-prone NHEJ pathway. In contrast, low-fidelity HR results from a lower NADH/NAD+

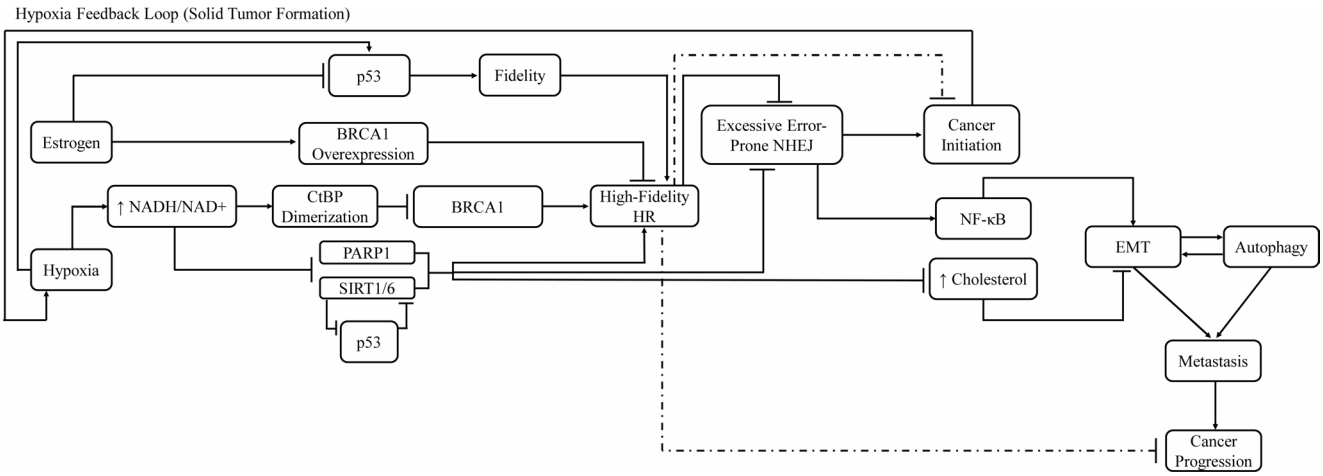

Fig. 6 Role of different molecular players in breast cancer initiation and progression. High-fidelity HR is impaired by estrogen signaling and hypoxia-induced metabolic changes (i.e., increased NADH/NAD+ ratio) that reroute DSB repair toward error-prone NHEJ. Cancer may initiate with this repair dysfunction, which sets off a cascade of NF-κB activation, EMT, and autophagy; all of which work together to support tumor progression and metastasis. The figure was created in Microsoft PowerPoint

ratio, as a lower redox ratio inhibits p53. Thus, deviations from the normal redox ratio may trigger genomic instability.

Estrogen alters normal BRCA1 dynamics by relieving CtBP-mediated repression. Further, by dysregulating BRCA1 balance, elevating DNA-PK activity, and generating free radicals that damage DNA, estrogen drives a shift in DSB repair toward NHEJ and initiates breast cancer development. Furthermore, although breast cancer cells that express ER generally retain wild-type p53, estrogen induces cytoplasmic translocation of p53. Nuclear expulsion of p53 compromises HR fidelity. Thus, BRCA1 expression, altered NADH/NAD+ ratio, and estrogen are the major drivers of breast cancer initiation.

On the other hand, in the context of breast cancer progression, the CtBP-ZEB1 corepressor complex reduces cholesterol synthesis by repressing SREBP2, thereby allowing TGF-β to induce EMT in cancer cells, which promotes their metastasis. Furthermore, SIRT6 inhibits SREBP2, promoting EMT. Cancer cells undergoing EMT require autophagy for survival. SIRT6 activates AMPK, which boosts the cell's energy reserves by stimulating ATP-producing catabolic processes and suppressing ATP-demanding anabolic processes. Thus, AMPK helps preserve metabolic balance and supports autophagy, both of which are essential during EMT and metastasis. Genomic instability is a key driver of cancer metastasis in the context of innate immunity. Under hypoxic conditions, an increase in the redox ratio and the dimerization of CtBP shift double-strand break repair toward the less accurate NHEJ pathway and stimulate innate immunity via NF-κB. Thus, cancer cells induce EMT through the CtBP-ZEB1 and SIRT6 pathways, activate survival-promoting autophagy via the SIRT6-AMPK pathway, induce genomic instability by increasing the NADH/NAD+ ratio under hypoxia, and co-opt innate immunity by activating NF-κB, which are key processes required for metastasis.

As a summary (Fig. 6), estrogen causes overexpression of BRCA1 while a higher NADH/NAD+ ratio causes repression of BRCA1. Both conditions impair HR and promote excessive NHEJ, which may lead to cancer initiation (Fig. 6). Similarly, a higher NADH/NAD+ inhibits SIRT1/6 and PARP1, impairing HR and promoting excessive NHEJ, which may lead to cancer initiation (Fig. 6). Cancer initiation leads to hypoxia, which increases the NADH/NAD+, reinforcing excessive NHEJ. Further, the higher NADH/NAD+ ratio in solid tumor increases cholesterol by inhibiting SIRT6, while genomic instability caused by the excessive NHEJ activates NF-κB, leading to EMT, which activates autophagy, leading to metastasis and cancer progression (Fig. 6). Furthermore, estrogen inhibits p53, leading to low fidelity HR, while a higher NADH/NAD+ ratio activates p53, inhibiting HR, contributing to cancer initiation and progression, which are inhibited by high fidelity HR (Fig. 6).

Perspective

Although significant progress has been made in deciphering the complex functions of CtBP in breast cancer, several challenges and opportunities remain. In this study, we emphasized that several aspects of cancer initiation and progression are linked. For example, we linked the metabolic redox ratio to BRCA1 and genomic instability via the corepressor CtBP. Furthermore, we linked CtBP with autophagy, inflammation, EMT, and NF-κB-mediated innate immunity. The dual nature of CtBP as a transcriptional co-repressor

and metabolic sensor indicates that its functions are highly complex and are involved in both cancer initiation and progression. Integrating CtBP-targeting agents with inhibitors of major oncogenic pathways, including Wnt, Notch, NF-κB, and autophagy, could yield synergistic therapies to overcome resistance and potentially enhance the response to breast cancer treatment.

The key aspects of designing and optimizing CtBP inhibitors will be driven by computational approaches, particularly molecular docking and molecular dynamics (MD) simulations. Docking methods are useful for quickly predicting the binding orientations and affinities of inhibitors towards the target protein, whereas MD simulations can provide important information on the stability and conformational changes of CtBP-inhibitor complexes under physiologically relevant conditions. The combination of these tools can accelerate lead compound identification and inform rational drug design strategies. Furthermore, combining these computational tools with experimental validation of cancer models, such as patient-derived organoids, could bridge the gap between simple mechanistic research and clinical application. Finally, the application of interdisciplinary approaches that integrate structural biology and computational modeling can help establish CtBP inhibition as a practical alternative treatment of breast cancer.

Methods

In this study, we conducted a systematic literature search of the PubMed and Google Scholar databases using the following keywords: CtBP, NADH, BRCA1, homologous recombination, non-homologous end joining, estrogen, p53, DNA-PK, SIRT6, SIRT1, EMT, AMPK, autophagy, NF-κB, hypoxia, and breast cancer metastasis. We have searched for English-language research articles, with a focus on recent publications. We have included all the foundational studies found necessary for the context. Further, we have included only the published and peer-reviewed research and review articles relevant to our study, specifically focused on topics such as pathway choice in DNA DSB repair, the effect of metabolic shift (NADH/NAD+ ratio), the role of CtBP, the crosstalk between estrogen and p53, and the involvement of SIRT1/6 and NF-κB in breast cancer progression, autophagy, and EMT.

We have excluded all non-peer-reviewed articles, such as preprints, brief communications, and abstract-only publications. Further, we have excluded all such articles that lack direct relevance to our review.

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Declarations

Competing interests The authors declare no competing interests.

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